(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 December 2000 (14.12.2000)

PCT

(10) International Publication Number
WO 00/74662 A2

(51) International Patent Classification[7]: A61K 31/00

(21) International Application Number: PCT/GB00/02048

(22) International Filing Date: 6 June 2000 (06.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/137,699 7 June 1999 (07.06.1999) US
0007321.3 27 March 2000 (27.03.2000) GB

(71) Applicant (*for all designated States except US*): UNIVERSITY OF SHEFFIELD [GB/GB]; Western Bank, Sheffield S10 2TN (GB).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): BUTTLE, David [GB/GB]; Unviersity of Sheffield, Medical School, Beech Hill Road, Sheffield S10 2R (GB). ADCOCKS, Clair [GB/GB]; University of Sheffield, Medical School, Beech Hill Road, Sheffield S10 2R (GB). COLLIN, Peter [US/US]; P.O. Box 151, Stonington, ME 04681 (US).

(74) Agent: HARRISON GODDARD FOOTE; Tower House, Merrion Way, Leed LS2 8PA (GB).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/74662 A2

(54) Title: ARTHRITIS TREATMENT

(57) Abstract: The invention relates to the use of catechins in the treatment of various forms of arthritis, including the use of combinations of catechins and other anti-arthritic agents in said treatment; medicaments and compositions for use in said treatment; and methods to identify agents with anti-arthritic properties.

Arthritis Treatment

The invention relates to the use of catechins, or varients thereof, in the treatment of all forms of arthritis.

Green tea is a very common drink in the Far East and its beneficial health properties have been recognised for many hundreds of years. Green tea has therefore been the subject of extensive research to identify the active agents responsible for said beneficial health effects. Green tea is a complex mixture of naturally occurring plant extracts. A group of these is variously described as flavanols, catechins or proanthocyanidins. The commonest of these in green tea are: epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG), please see Figure 1.

Catechins have been reported to alleviate a number of clinical conditions. These include stroke and cerebral haemorrhage (Sato *et al*, 1989), cardiovascular and liver diseases (Imai & Nakachi, 1995), bacterial infections (Ikigai *et al.*, 1993) and stomach ulcers (Murakami *et al.*, 1992). Catechins have also been shown to inhibit the release of histamine and leukotriene which indicates that catechins may have benefit with regard to the treatment of various allergic disorders (Matsuo *et al*, 1997).

In addition, catechins have also been shown to inhibit the lipopolysaccharide (LPS) induced release of tumour necrosis factor α (TNFα) production at the level of both transcription and release from activated macrophages and therefore may have use in the inhibition of inflammation (Yang *et al.*, 1998).

Catechins have been shown to regulate cholesterol levels; to have anti-mutagenic properties; to reduce blood pressure; to inhibit the effects of various agents on the liver and can also protect teeth from decay. Clearly catechins have a number of beneficial health effects which are well known in the art.

By far the most interest has been in the anti-cancer effects of catechins. Epidemiological studies have suggested that consumption of green tea may help to

prevent cancers in humans (Yang *et al.*, 1993), and at least 17 clinical studies have been published, for example Gao *et al.*, 1994.

We have discovered a further beneficial therapeutic effect of catechins which relates to the treatment of arthritis.

Catechin is a generic name for a group of compounds based on the structure catechin itself, (3',3',4',5,7-flavanpentol), please see Figure 1.

We have recently discovered that catechins are chondroprotective, that is they inhibit the breakdown of cartilage extracellular matrix. EGCG, ECG and EC, at a dose of 20μM significantly inhibited proteoglycan loss resulting from treatment of bovine cartilage explants with recombinant human interleukin 1α (rhIL-α). When recombinant human TNFα (rhTNFα) provided the catabolic stimulus, EGCG produced a dose-response curve for inhibition of proteoglycan loss, with about 50% inhibition being achieved at a concentration of 2μM, please see Figure 2. In these experiments, EGCG showed no toxic effects. At 218μM EGCG was without effect on lactate production by the explants. Also at an effective concentration range of 2μM and 20μM, ^{35}S incorporation as a measure of proteoglycan synthesis was unaffected. In addition, the inhibitory effect of EGCG on TNF-mediated proteoglycan breakdown was fully reversible following removal of the catechin from the explants (not shown).

EGCG is reported to inhibit TNFα synthesis (Yang *et al.*, 1998; Suganuma *et al.*, 1996), which may provide the basis for its anti-inflammatory effects. We have confirmed that EGCG does indeed have this important property. At a concentration of 20μM it inhibited bacterial lipopolysaccharide-stimulated TNFα synthesis from blood samples from two different volunteers by 66% and 30% using two different commercially available ELISA kits. However, this cannot provide the mechanism for inhibition of cartilage breakdown as large amounts of exogenous cytokine are added to the cartilage culture in experiments such as those shown in Figure 2.

This leads to the conclusion that catechins have two distinct properties that should be beneficial to arthritis sufferers, an anti-inflammatory and a distinct chondroprotective effect.

According to the first aspect of the invention there is provided the use of at least one catechin for the manufacture of a medicament for the treatment of arthritis.

In a preferred embodiment of the invention said catechin is selected from: (+)epicatechin, (+)catechin, (-)epicatechin; (-)catechin, (-)epigallocatechin; (-)gallocatechin; (-)epicatechin gallate; (-)catechin gallate; (-)epigallocatechin gallate; (-)gallocatechin gallate; or varients thereof.

In a further preferred embodiment of the invention said catechin is epigallocatechin gallate.

In yet a further preferred embodiment of the invention said catechin is epicatechin gallate.

In a yet further preferred embodiment of the invention said medicament is for the treatment of arthritic conditions selected from: osteoarthritis, rheumatoid arthritis; inflammatory arthritis; osteochondritis; acute pyrophosphate arthritis; reactive arthritis; psoriatic arthritis; juvenile arthritis; lupus erythematosus; Sjogren's syndrome; relapsing polychondritis; ankylosing spondylitis; psoriatic arthritis; MSUM (gout); CPDD (pseudogout, chondrocalcinosis); chondrolysis; bursitis.

In yet a still further preferred embodiment of the invention said medicament is for the treatment of osteoarthritis.

In yet a still further preferred embodiment of the invention said medicament is for the treatment of rheumatoid arthritis.

In yet still a further preferred embodiment of the invention said medicament is for the use in prophylactic treatment of arthritis. Ideally said prophylactic treatment is for animals with a genetic predisposition to arthritis, preferably osteoarthritis.

Alternatively or preferably, said prophylactic treatment is to protect animals with an increased probability of developing arthritis due to joint damage (eg. cruciate ligament damage). It is well known in the art that individuals which suffer joint damage have an increased incidence of arthritis (Price *et al.*, 1999).

According to the second aspect of the invention there is provided a therapeutic composition comprising at least one catechin and at least one anti-arthritic agent or biopolymer. Preferably said composition is for use in the manufacture of a medicament for the treatment of arthritis, ideally osteoarthritis. More ideally still said anti-arthritic agent is hyaluronic acid, or varients thereof.

Alternatively or preferably said anti-arthritic agent is glucosamine, or varients thereof, preferably glucosamine sulphate. It is reported that glucosamine is an effective treatment of arthritic conditions (MacCarty, 1994; MacCarty, 1998). Current opinion suggests that glucosamine stimulates the production of glycosaminoglycans, such as hyaluronic acid in joints.

Hyaluronic acid is a polymer of *N*-acetyl glucosamine and glucuronic acid molecules and is well known to have anti-arthritic properties (Balazs, 1968; Gibbs *et al.*, 1968; Balazs & Gibbs., 1970; Rydell *et al.*, 1970; Weiss *et al.*, 1981; Denlinger, 1982; Balazs,1982; Balazs & Denlinger, 1985; Weiss. & Balazs, 1987; Balazs & Denlinger, 1989; McCain *et al.*, 1989; Adams, 1993; Balazs & Denlinger, 1993; Moreland *et al.*, 1993; Peyron, 1993a; Peyron, 1993b; Scale *et al.*, 1994; Adams *et al.*, 1995; Band *et al.*, 1995; Baker, 1997 Balazs & Larsen, 1997; Adams, 1998; Denlinger, 1998; Dickson & Hosie, 1998; Estey, 1998; Wobig *et al.*, 1998 and Peyron, 1999.

It is naturally occurring in all mammals in a variety of tissues (eg. synovial fluid, vitreous humour) and some bacterial species. Hyaluronic acid can vary in molecular mass from 50kDa to 8 x 10^3 kDa and forms highly viscous solutions. Methods to

prepare pure samples, which are non-inflammatory, are well known in the art. For example, EP 0239335 & US 4879375 disclose methods to prepare highly pure fractions of hyaluronic acid which purport to be non-inflammatory. Hyaluronic acid is known to have a variety of therapeutic effects. For example, and not by the way of limitation, the treatment of various skin disorders, (described in US 5914322) and the treatment of articular degeneration as a consequence of corticosteroid treatment, (described in US 4801619). Hyaluronic acid, and the like, provide visco-supplementation and/or viscolubrication (Balazs & Denlinger, 1993; Peyron, 1993a; Scale *et al*., 1994; Lussier *et al*., 1996) to replace fragmented hylauronic acid as a consequence of arthritic disease.

In a preferred embodiment of the invention said catechin and anti-arthritic agent are administered as a simple admixture. Alternatively, said catechin and anti-arthritic agent are crosslinked, coupled or associated together.

It is possible to crosslink or conjugate hyaluronic acid to various therapeutic molecules. For example, EP 0296740 describes the production of hyaluronic acid conjugates. Hyaluronic acid has a number of free hydroxyl and carboxyl groups to which catechins may be crosslinked or coupled either directly or via crosslinking agents. Alternatively, hyaluronic acid and a catechin are encapsulated within a liposome preparation as detailed below.

In an alternative embodiment of the invention said catechin is crosslinked, coupled or associated with hyaluronic acid.

According to a third aspect of the invention there is provided a method to crosslink or couple at least one catechin to at least one anti-arthritic agent comprising:

i) providing at least one catechin and at least one anti-arthritic agent;

ii) providing conditions conducive to the crosslinking or coupling of said catechin to said agent; and, optimally

iii) purifying the crosslinked or coupled complex from the reaction mixture.

In a preferred method of the invention said anti-arthritic agent is hyaluronic acid and said catechin is selected from: (+)catechin; (+)epicatechin; (-)catechin; (-)epigallocatechin; (-)gallocatechin; (-)epicatechin gallate; (-)catechin gallate; (-)epigallocatechin gallate; (-)gallocatechin gallate; or varient thereof.

According to a yet further aspect of the invention there is provided a method of treating an arthritic condition comprising administering to an animal a pharmacologically effective amount of the therapeutic composition/medicament according to the invention.

In a preferred method of the invention said arthritic condition is selected from; osteoarthritis; rheumatoid arthritis; osteochondritis; acute pyrophosphate arthritis; reactive arthritis; psoriatic arthritis; juvenile arthritis; lupus erythematosus; Sjögren's syndrome; relapsing polychondritis; ankylosing spondylitis; psoriatic arthritis; MSUM (gout); CPPD (pseudogout, chondrocalcinosis); chondrolysis; bursitis.

In a still preferred method of the invention said arthritic condition is osteoarthritis.

In a yet still further preferred method of the invention said arthritic condition is rheumatoid arthritis.

It will be apparent to one skilled in the art that the therapeutic compositions/medicaments can be formulated in a variety of ways to facilitate delivery. For example, liposomal compositions may be usefully employed to deliver said compositions/medicaments.

Liposomes are lipid based vesicles which encapsulate a selected therapeutic agent which is then introduced into a patient. Typically, the liposome is manufactured either from pure phospholipid or a mixture of phospholipid and phosphoglyceride.

Typically liposomes can be manufactured with diameters of less than 200nm, which enables them to be intravenously injected and able to pass through the pulmonary capillary bed. Furthermore the biochemical nature of the liposomes confers permeability across blood vessel membranes to gain access to selected tissues. Liposomes do have a relatively short half-life. So-called STEALTH® liposomes have been developed which comprise liposomes coated in polyethylene glycol (PEG). The PEG treated liposomes have a significantly increased half-life when administered intravenously to a patient. In addition STEALTH® liposomes show reduced uptake in the reticuloendothelial system and enhanced accumulation in selected tissues. In addition, so called immuno-liposomes have been developed which combine lipid based vesicles with an antibody or antibodies, to increase the specificity of the delivery of the therapeutic composition/medicament to a selected cell/tissue.

The use of liposomes as a delivery means is described in US 5580575 and US 5542935.

It will be apparent to one skilled in the art that the compositions/medicaments can be imbided or provided in the form of an oral or nasal spray, an aerosol, suspension, emulsion, and/or eye drop. Alternatively the medicament may be provided in tablet form. Alternative delivery means include inhalers or nebulisers.

Alternatively or preferably the medicament can be delivered by direct injection into a joint. It is envisioned that the compositions/medicaments be delivered intravenously, intramuscularly, subcutaneously or topically. Further still, the medicament may be taken rectally.

It will also be apparent that compositions/medicaments are effective at preventing and/or alleviating arthritic conditions in animals other than humans, for example and not by way or limitation, family pets, livestock, horses.

According to a fourth aspect of the invention there is provided a method to screen for agents with anti-arthritic properties.

In a preferred method of the invention said method comprises:

i) providing a cartilage sample.

ii) addition of an effective amount of at least one agent to be tested.

iii) addition of at least one pro-inflammatory cytokine; and

iv) monitoring at least one molecule indicative of cartilage breakdown.

In a further preferred embodiment of the invention said inflammatory cytokine is selected from:interleukin-1α; interleukin-1β; oncostatin M; tumour necrosis factor-α. In a preferred method of the invention said method comprises:

i) providing a cartilage sample;
ii) addition of an effective amount of at least one agent to be tested;
iii) addition of one vitamin A metabolite; and
iv) monitoring at least one molecule indicative of cartilage breakdown.

Preferably said vitamin A metabolite is all-*trans*-retinoic acid.

It will be apparent to one skilled in the art that methods to monitor cartilage degradation are well known and are herein described.

According to a further aspect of the invention there is provided an agent identified by the screening method of the invention.

It will also be apparent to one skilled in the art that catechins disclosed above can either be isolated from natural plant sources, eg *Camellia sinensis*; *Uncarcia gambir* or can be synthesised in the laboratory using methods well known in the art.

An embodiment of the invention will now be described, by example only, and or with reference to the following Tables and Figures:

Table 1 represents the inhibitory effects of catechins on cytokine or vitamin A metabolite-stimulated cartilage breakdown using (i) nasal cartilage explants and (ii) articular cartilage explants;

Table 2 represents the inhibitory effects of EGCG and ECG on human osteoarthritic cartilage explants;

Table 3 represents the inhibitory effects of EGCG and ECG on the degradation of proteoglycan of human rheumatoid knee cartilage;

Table 4 represents the inhibitory effects of EGCG and ECG on the degradation of proteoglycan of human non-arthritic cartilage;

Tables 5 and 6 represent the inhibitory effects of EGCG, ECG, EC and EGC on type II collagen degradation in bovine nasal cartilage explants stimulated with rhILα;

Table 7 demonstrates lactate production by the explants over the latter part of a 28-day period;

Table 8 represents the inhibitory effects of EGCG on the synthesis of human TNFα;

Figure 1a and 1b represents the chemical structures of a selection of catechins and variants thereof;

Figure 2 represents the dose-response for EGCG inhibitory activity of TNFα induced cartilage proteoglycan breakdown; and

Figure 3 represents the macroscopic changes in bovine nasal cartilage explants cultured in the presence or absence of rhIL-1α, EC and EGC for a 28 day period.

Introduction.

Cartilage proteoglycan degradation in the bovine system was stimulated with the proinflammatory cytokines interleukin1α (IL1α) or tumour necrosis factor α (TNFα) or with the vitamin A derivative, all-*trans* retinoic acid (Ret). The degradatory process was initiated in the human cartilage using a combination of interleukin1β (IL1β) and TNFα. These are standard *in vitro* models for the breakdown of cartilage proteoglycan components that occurs in arthritis (Bryson *et al.*, 1998; Ilic *et al.*, 1992).

Methods

The inhibition of cartilage proteoglycan degradation.

Bovine nasal septum and metacarpophalangeal cartilage was prepared as described by Buttle *et al.*, (1992). The nasal septum cartilage was removed using a post-mortem knife and the overlying membrane was discarded. The excised cartilage was wiped with isoprenyl-impregnated Azowipes, placed in a petri dish in the tissue culture hood and washed in sterile phosphate buffered saline (PBS). The cartilage was sliced using a scalpel blade into approximately 2mm x 3mm x 3cm slices, and from these slices discs were cut with the aid of a belt punch. About 200 discs (~3mm diam., 2mm thick) were obtained from each animal.

Slices of cartilage from the bovine metacarpophalangeal joint were dissected and cut into small pieces similar in size to the nasal explants. All bovine cartilage explants were cultured overnight in DMEM (Dulbecco's Modified Eagles Medium) containing newborn calf serum (NCS) (5%) and hydrocortisone (0.1 μg/ml) prior to the start of an experiment.

In different experiments human articular cartilage was obtained following various surgical procedures. The cartilage was dissected into slices and in some experiments the proteoglycan was biosynthetically labelled with 5 μCi/ml $^{35}SO_4$ in 5% NCS-containing DMEM for 5 days. The cartilage was then washed in 5% NCS-containing DMEM without radiolabel for 2 days prior to the start of an experiment.

The effect of EGCG and ECG on the degradation of the proteoglycan component of bovine and human cartilage.

Cartilage explants treated as described above were individually transferred into the wells of a 96-well plate and cultured for up to 9 days (medium change on days 3 and 6 or on day 4) in serum-free DMEM in the presence or absence of recombinant human IL1α (rhIL1α), recombinant human IL1β (rhIL1β), recombinant human TNFα (rhTNFα) and Ret, either singly or in combination. On average greater than 50% of the sulfated glycosaminoglycans (sGAGs) in the explants was released following stimulation, which was twice the basal release. Certain catechins were prepared as stock solutions in dimethyl sulfoxide (DMSO) and further diluted in DMSO or DMEM to give the appropriate final concentration in the culture medium and also a constant 1% (v/v) DMSO concentration.

sGAGs within the conditioned media and retained in the tissue (measured following papain digestion) were either determined by the dimethylmethylene blue assay (Farndale *et al.*, 1986) or by scintillation counting following labelling with $^{35}SO_4$ (Ilic *et al.*, 1995). Data were expressed as the percentage sGAG released from the tissue or as the percentage inhibition of sGAG release. The Mann-Whitney U test for unpaired, non-parametric data was used to determine the statistical significance of the results.

Type II collagen breakdown in rhIL-1α-stimulated bovine nasal cartilage explants.

In order to investigate the effect of catechins on type II collagen degradation, bovine nasal cartilage explant cultures were maintained for 28 days, with a twice-weekly medium change, in the presence or absence of rhIL-1α (4.5nM) and the catechins; EGCG, ECG, EC and EGCG at 20μM. At the completion of the experiment the type II collagen remaining in the cartilage residues was extracted by digestion with proteinase K (EC 3.4.21.64) at 56°C for 15 hours. The extracts were assayed by inhibition enzyme-linked immunosorbent assay (ELISA) using a mouse IgG monoclonal antibody to denatured type II collagen, Col2-3/4m as previously

described (Hollander *et al.*, 1994). The amount of collagen released at each medium change throughout the culture period was calculated as a percentage of total collagen in each culture well (medium plus tissue residue).

Effect of catechins on proteoglycan synthesis.

Bovine nasal cartilage explants were obtained as described above. The rate of proteoglycan synthesis was assessed by measuring incorporation of ^{35}S from $^{35}SO_4$. Three groups were set up: (a) killed explants (freeze-thawed thrice), (b) explants cultured in serum-free DMEM alone, (c) explants cultured in serum-free DMEM and 2μM or 20 μM EGCG. All three groups were cultured in the presence of 5μCi $^{35}SO_4$/ml for 18 hours and ^{35}S incorporation was assessed as previously described by Buttle *et al.* (1993).

Lactate test for assessment of chondrocyte metabolic activity.

Chondrocytes respire anaerobically (Stefanovic-Racic *et al.*, 1994). A measure of the toxicity of a compound can therefore be made by determining the levels of lactate in the conditioned media by using the lactate oxidase/peroxidase method with a kit from Sigma Chemical Co.

The effect of catechins on TNFα production by human peripheral blood cells.

Peripheral blood was taken from volunteers and 50 iu/ml heparin was added. The blood was diluted 1:6 with serum-free DMEM and incubated for 4 hours at 37°C in a water bath in the presence or absence of 1μg/ml lipopolysaccharide (LPS) (*E.coli*) and EGCG (20 μM). The blood was freeze-thawed thrice at –40°C and then centrifuged at 1000 rpm for 5 min to remove cell debris. A TNFα ELISA was then performed on the supernatant following the manufacturers' instructions, either using a kit from R&D systems or from Diaclone Research.

RESULTS

Inhibition of basal and stimulated levels of bovine nasal and articular cartilage proteoglycan degradation by the catechins at 20 μM.

As shown in Table 1(i), EGCG significantly inhibited rhTNFα-stimulated cartilage proteoglycan degradation in a bovine nasal cartilage explant model, but no significant effect was observed for the basal, rhIL1α-, or Ret-stimulated release. Both ECG and EC significantly inhibited IL1α-stimulated degradation but not rhTNFα- or Ret-stimulated breakdown.

In the bovine articular explant model (Table 1 ii), EGCG again potently inhibited the rhTNFα-stimulated response, whilst also inhibiting, but to a lesser degree the basal, rhIL-1α and Ret response. Both the rhIL-α and Ret responses were more potently inhibited by ECG.

Dose-response for the inhibition of rhTNFα-stimulated bovine cartilage proteoglycan degradation by EGCG.

In view of the potent inhibition of rhTNFα-stimulated cartilage proteoglycan degradation by 20μM EGCG, a dose-response curve was constructed (Fig. 2). Inhibition reached statistical significance at 2 μM (47% inhibition) and increased to 84% and 138% inhibition at 20μM and 200μM respectively. Where inhibition exceeded 100% some of the basal breakdown as well as rhTNFα-stimulated breakdown is indicated.

Influence of catechins on the lactate output of chondrocytes.

EGCG at 200 μM was shown not to be toxic over a 5 day period by measurement of lactate levels in the conditioned media of bovine nasal cartilage explants stimulated with rhTNFα. Explants cultured in DMEM alone produced 896 μg lactate/explant and in the presence of EGCG, this was 1040 μg lactate/explant. When explants were

cultured in the presence of rhTNFα a total of 1248 μg lactate/explant was produced, decreasing slightly to 896 μg when cultured in the presence of EGCG. These results demonstrate that over a 5-day period EGCG did not have a marked effect on lactate output by chondrocytes.

The effect of EGCG on the synthesis of bovine nasal cartilage proteoglycan.

EGCG at 2 μM had no significant effect on proteoglycan synthesis in bovine nasal cartilage explants, giving a non-significant stimulation (12% ± 15%), whilst at 20 μM it gave a non-significant inhibition of 32% ± 8% (2 animals, n=8 explants/animal).

The effect of catechins on human cartilage proteoglycan degradation.

As shown in Tables 2, 3 and 4, ECG (20 μM) produced significant inhibition of proteoglycan breakdown from human cartilage from osteoarthritic, rheumatoid and non-arthritic joints. EGCG inhibited basal levels of proteoglycan loss from osteoarthritic cartilage (Table 2).

The effect of catechins on collagen degradation in rhIL-1α-stimulated cartilage proteoglycan degradation.

As Tables 5 and 6 demonstrate, the culture of bovine nasal cartilage explants for 28 days in the presence of rhIL1α resulted in the almost complete degradation of the explants (shown visually in Fig. 3), with almost total release of type II collagen by IL1α. EGCG, ECG and EGC (20 μM) significantly reduced this degradation (Table 6) with the percentage type II collagen released from the explants decreasing by more than 50% in all three cases. As Table 7 shows, the culture of bovine nasal cartilage explants for a 28-day period in the presence of rhIL1α and EGCG or ECG was not associated with any toxic effects, as determined by the levels of lactate in the conditioned media over the 17-20 day and 24-28 day culture periods.

The effect of the catechins on TNFα synthesis by human peripheral blood cells.

EGCG has been reported to inhibit TNFα synthesis in a human stomach cancer cell line KATO III (Okabe *et al.*, 1999) and in BALB/3T3 cells (Suganuma *et al.*, 1999). We tested the effects of EGCG (20 μM) on LPS-stimulated TNFα synthesis by whole blood. In separate experiments using blood samples from different volunteers and ELISA kits from different sources, we confirmed the inhibition of TNFα synthesis by EGCG (Table 8).

REFERENCES

Adams, M.E. An analysis of clinical studies of the use of crosslinked hyaluronan, Hylan, in the treatment of osteoarthritis. J. Rheumatol. 20 (Suppl. 39):16-18, 1993.

Adams, M.E., Atkinson, M.H., Lussier, A., Schulz, J.I., Siminovitch, K.A., Wade, J.P., and Zummer, M. (1995). The role of viscosupplementation with Hylan G-F 20 (Synvisc®) in the treatment of osteoarthritis of the knee. Osteoarthritis and Cart. 3:213-226, 1995.

Adams, M.E. Viscosupplementation as articular therapy. In The Chemistry, Biology and Medical Applications of hyaluronan and its derivatives (Proceedings of Wenner-Gren Foundation International Symposium held in honor of Endre A. Balazs, September 18-21, 1996 Stockholm, Sweden) (Ed. Laurent T., Portland Press, London), 243-253.

Baker, D.E. Pharmacy and Therapeutics Review. The Formulary, December 1997.

Balazs, E.A. Viscoelastic properties of hyaluronic acid and biological lubrication. Univ. Michigan. Med. Ctr. Journal, 255-259, 1968.

Balazs, E.A. and Gibbs, D.A. The rheological properties and biological function of hyaluronic acid. In Chemistry and Molecular Biology of the Intercellular Matrix (Ed. Balazs, E.A), Academic Press, London and New York, 1241-1254, 1970.

Balazs, E.A. The physical properties of synovial fluid and the special role of hyaluronic acid. In Disorders of the Knee, Second Edition, (Ed. Heflet, A.), J.B. Lippincott Company, Philadelphia, 61-74, 1982.

Balazs, E.A. and Denlinger, J.L. Sodium hyaluronate and joint function. J. Equine Vet. Sci. 5:217-228, 1985.

Balazs, E.A. and Denlinger, J.L. Clinical uses of hyaluronan. In the Biology of Hyaluronan (Ciba Foundation Symposium #143) (eds. Evered, D. and Whelan, J.), John Wiley & Sons, Chichester and New York, 265-280, 1989.

Balazs, E.A. and Denlinger, J.L. Viscosupplementation: A new concept in the treatment of osteoarthritis. J. Rheumatol. 20 (Suppl. 39):3-9, 1993.

Balazs, E.A. and Larsen, N.E. Safety data from seven clinical studies. Biomatrix Report BXR 9009, 1997.

Band, P., Goldman, A., Barbone, A., Reiner, K., and Balazs, E.A. Intra-articular distribution and residence time of Hylan polymers. Materials Research Society, Spring Meeting, April 17-21, 1995, San Francisco, CA 433 (abstract).

Bryson, H., Bunning, R.A.D., Feltell, R., Kam, C.-M., Kerrigan, J., Powers, J.C., and Buttle, D.J. A serine proteinase inactivator inhibits chondrocyte-mediated cartilage proteoglycan breakdown occurring in response to proinflammatory cytokines. Arch. Biochem. Biophys.355 (1): 15-25, 1998.

Buttle, D.J., Saklatvala, J., Tamai, M., and Barrett, A.J. Inhibition of interleukin-1-stimulated cartilage proteoglycan degradation by a lipophilic inactivator of cysteine endopeptidases. Biochem. J. 281:175-177, 1992.

Buttle D.J., Saklatvala, J., and Barrett, A.J. The inhibition of interleukin-1-stimulated cartilage proteoglycan degradation by cysteine endopeptidase inactivators. Agents Actions Suppl. 39:161-165, 1993.

Denlinger, J.L. Metabolism of sodium hyaluronate in articular and ocular tissues. Ph.D. thesis, Université des Sciences et Techniques de Lille, Lille, France, 1982.

Denlinger, J.L. Hyaluronan and its derivatives as viscoelastics in medicine. In The Chemistry, Biology and Medical Applications of Hyaluronan and its Derivatives (Proceedings of the Wener-Gren Foundation International Symposium held in honor

of Endre A. Balazs, September 18-21, 1996, Stockholm, Sweden) (Ed. Laurent, T.), Portland Press, London, 235-242.

Dickson, D.J. and Hosie, G. Double-blind, double-control comparison of viscosupplementation with Hylan G-F 20 (Synvisc®) against dicolfenac and control in knee osteoarthritis. American College of Rheumatology, 62nd National Scientific Meeting, Nov 8-12, 1998, San Diego, CA. Arthritis Rheum. 41 (9), S197 (abstract).

Estey, D. Viscosupplementation - A new treatment for patients with osteoarthritis. Surgical Physician Assistant, 19-23, 1998.

Farndale, R.W., Buttle, D.J., and Barrett, A.J. Improved quantitation and discrimination of sulfated glycosaminoglycans by use of dimethylmethylene blue. Biochim. Biophys. Acta 883: 173-177, 1986.

Gibbs, D.A., Merrill, E.W., Smith, K.A., and Balazs, E.A. The rheology of hyaluronic acid. Biopolymers, 6: 777-791, 1968.

Hollander, A.P., Heathfield, T.F., Webber, C, Iwata, Y., Bourne, R., Rorabeck, C., and Poole, A.R. Increased damage to type II collagen in osteoarthritic articular cartilage detected by a new immunoassay. J. Clin. Invest. 93:1722-1732, 1994.

Ikigai, H., Nakae, T., Hara, Y. and Shimamura, T. Bactericidal catechins damage the lipid bilayer. Biochim. Biophys. Acta 1147: 132-136, 1993.

Ilic, M.Z., Handley, C.J., Robinson, H.C. and Mok, T.M. Mechanism of catabolism of aggrecan by articular cartilage. Arch. Biochem. Biophys. 294: 115-122, 1992.

Ilic, M.Z., Haynes, S.R., Winter, G.M. and Handley, C.J. Kinetics of release of aggrecan from explant cultures of bovine cartilage from different sources and from animals of different ages. Acta Orthop. Scand. 66 (Suppl. 266): 33-37.

Imai, K. and Nakachi, K. Cross sectional study of effects of drinking green tea on cardiovascular and liver diseases. Br. Med. 310: 693-696, 1995.

Lussier, A., Cividino, A.A., McFarlane, C.A., Olszynski, W.P., Potasher,W.J., and de Médicis, R. Viscosupplementation with Hylan for the treatment of osteoarthritis: findings from clinical practice in Canada. J. Rheumatol. 23:1579-1585, 1996.

Matsuo, N., Yamada, K., Shoji, K., Mori, M. and Sugano, M. Effect of tea polyphenols on histamine release from rat basophilic leukemia (RBL-2H3) cells: the structure-inhibitory activity relationship. Allergy 52:58-64, 1997.

MacCarty, M.F. Enhanced synovial production of hyaluronic acid may explain rapid clinical response to high glucosamine in osteoarthritis. (1994). Med. Hypotheses 42: 323-327, 1994.

MacCarty, M.F. The neglect of glucosamine as a treatment for osteoarthritis - a personal perspective. Med. Hypotheses 50: 507-510, 1998.

McCain, J.P. Balazs, E.A. and de la Rua, H. Preliminary studies on the use of a viscoelastic solution in arthroscopic surgery of the temporomandibular joint. J. Oral Maxillofac. Surg. 47:1161-1168, 1989.

Moreland, L.W., Arnold, W.J., Saway, A., Savory, C. and Sikes, D. Efficacy and safety of intra-articular Hylan G-F 20 (Synvisc®), a viscoelastic derivative of hyaluronan, in patients with osteoarthritis of the knee. American College of Rheumatology, 57th Annual Scientific Meeting, San Antonio, TX, Nov. 7-11, 1993, 165 (abstract).

Murakami, S., Muramatsu, M. and Otomo, S. Gastric H^+, K^+-ATPase inhibition by catechins. J. Pharm. Pharmacol. 44: 926-928, 1992.

Okabe, S., Ochiai, Y., Aida, M., Park, K., Kim, S-J., Nomura, T., Suganuma, M., and Fujiki, H. Mechanistic aspects of green tea as a cancer preventive. Effect of

components on human stomach cancer cell lines. Jpn. J. Cancer. Res. 90: 733-739, 1999.

Peyron, J.G. A new approach to the treatment of osteoarthritis: viscosupplementation. Osteoarthritis Cart. 1:85-87, 1993a.

Peyron, J.G. Intra-articular hyaluronan injections in the treatment of osteoarthritis: state-of-the-art review. J. Rheumatol. 20 (Suppl. 39):10-15, 1993b.

Peyron, J.G. Viscosupplementation for the treatment of osteoarthritis of the knee with hyaluronan and Hylans: rationale and state of the art. In Advances in Osteoarthritis (Eds. Tanaka, S and Hamanishi, C.), Springer-Verlag, Tokyo, 213-236,1999

Price, J.S., Wang-Weigand, S., Bohne, R., Kozaci, L.D., and Hollander, A.P. Retinoic acid-induced type II collagen degradation does not correlate with matrix metalloproteinase activity in cartilage explant cultures. Arthritis Rheum. 42: 137-147, 1999.

Rydell, N.W. Butler. J., and Balazs, E.A. Hyaluronic acid in synovial fluid. VI. Effect of intra-articular injection of hyaluronic acid on the clinical symptoms of arthritis in track horses. Acta. Vet. Scand. 11: 139-155, 1970

Sato, Y., Nakatsuka, H., Watananbe, T., Hisamichi, S., Shimizu, H., Fujisaku, S., Ichinowatari, Y., Ida, Y., Suda, S., Kato, K. and Ikeda, M. Possible contribution of green tea drinking habits to the prevention of stroke. Tohoku J. Exp. Med. 157: 337-343, 1989.

Scale, D., Wobig, M., and Wolpert, W. Viscosupplementation of osteoarthritic knees with Hylan: a treatment schedule study. Curr. Therap. Res. 55:220-232, 1994.

Stefanoviv-Racic, M., Stadler, J., Georgescu, H.I., and Evans, C.H. Nitric oxide and energy production in articular chondrocytes. J. Cell. Physiol. 159: 274-280, 1994.

Suganuma, M. Okabe, S., Sueoka, E., Iida, N., Komori, A. Kim, S.-J. and Fjiki, H. A. New process of cancer prevention mediated through inhibition of tumour necrosis factor α expression. Cancer Res. 56: 3711-3715, 1996.

Suganuma, M., Okabe, S., Sueoka, N., Sueoka, E., Matsuyama, S., Nakachi, K., and Fujiki, H. Green tea and cancer prevention. Mutat. Res. 428: 339-344, 1999.

Weiss, C., Balazs, E.A., St Onge, R. and Denlinger, J.L. Clinical studies of the intra-articular injection of Healon® (sodium hyaluronate) in the treatment of osteoarthritis of human knees. In Seminars in Arthritis and Rheumatism, Vol 11, (Ed. Talbott, J.H.) Grune and Stratton, New York, 143-144, 1981.

Weiss, C. and Balazs, E.A. Arthroscopic viscosurgery. Arthroscopy 3:138-139, 1987.

Wobig, M., Dickhut, A., Maier, R., and Vetter, G. Viscosupplementation with Hylan G-F 20: A 26-week controlled trial of efficacy and safety in the osteoarthritic knee. Clin. Therap. 20 (3):410-423, 1998.

Yang, C.S., and Wang, A.-Y. Tea and cancer. J. Natl. Cancer Inst. 85: 1038-1049, 1993.

Yang. F., de Villiers, W.J.S., McClain, C.J., and Varilek, G.W. Green tea polyphenols block endotoxin-induced tumour necrosis factor-production and lethality in a mouse model. J. Nutr. 128: 2334-2340, 1998.

Table 1

The inhibition of cartilage proteoglycan degradation in bovine nasal and articular cartilage explants by the catechins (20μM).

(i) Nasal cartilage explants

% Inhibition of stimulated sGAG release from cartilage

	EGCG	ECG	EC	EGC
Control	(d)5±5	(c)-7±13	(c)5±10	(c)-6±9
IL-1α	(a)13±7	(a)27±4***	(a)17±4**	(a)3±4
TNFα	(a)92±6***	(a)-25±36	(a)33±16	(a)-21±33
Ret	(b)-13±15	(b)7±22	(b)-34±20	(b) -23±22

(ii) Articular cartilage explants

% Inhibition of stimulated sGAG release from cartilage

	EGCG	ECG	EC	EGC
Control	(a)34±5***	(c)8±5	(c)10±8	(c)16±6
IL-1α	(a)33±7*	(c)51±12**	(c)13±11	(c)4±13
TNFα	(c)84±7***	(c)25±9*	(c)-6±7	(c)9±9
Ret	(b)27±10*	(a)39±13*	(a)2±9	(a)34±13

Table 1

Bovine nasal and articular cartilage explants were cultured for 5 days in serum-free DMEM in the presence or absence of rhIL-1α (0.3 nM nasal; 3nM articular), rhTNFα (3nM nasal; 6nM articular) or Ret (1μM) and in the presence or absence of the catechins EGCG, ECG, EC or EGC at a final concentration of 20 μM. Medium was changed on day 3. The degradation of cartilage proteoglycan was determined by measuring the sGAG released from the explants as a percentage of total sGAG using the DMB assay. The data are expressed as the mean percentage inhibition of stimulated or basal cartilage proteoglycan degradation ± s.e.m. [a] 4 animals, n=32; [b] 6 animals, n= 48; [c] 2 animals, n=16; [d] 7 animals, n=56. * $p<0.05$, ** $p<0.005$ and *** $p<0.0005$ as determined using the 2-tailed Mann-Whitney test for non-parametric data.

Table 2

The inhibition of cartilage proteoglycan degradation in human osteoarthritic cartilage explants by EGCG and ECG (20μM)

	% sGAG released
Control	23±2
EGCG	18±1 *
ECG	23±2
IL-1β/TNFα	30±2
IL-1β/TNFα + EGCG	27±2
IL-1β/TNFα + ECG	25±1 *

Human osteoarthritic articular cartilage explants were biosynthetically labelled for 5 days in 5%(v/v) newborn calf serum (NCS)-containing DMEM using $^{35}SO_4$ (5μCi/ml). The explants were washed for 2 days and then cultured for a further 9 days in the presence or absence of a combination of rhIL1β (3nM) and rhTNFα (6nM) and also in the presence or absence of the catechins EGCG or ECG at 20μM. Medium was changed on day 3 and day 6. The degradation of cartilage proteoglycan was determined by measuring the radiolabel released from the explants as a percentage of total radiolabel by quantifying the ^{35}S released into the culture medium and that retained in the tissue by use of a scintillation counter. The data are expressed as the mean percentage release ± s.e.m. *$p<0.05$, when the release of sGAG is compared between the groups cultured in the presence of the catechins to those cultured in their absence, as determined by the Mann Whitney U test for 2-tailed, non-parametric data.

Table 3

The inhibition of cartilage proteoglycan degradation in human rheumatoid cartilage explants by EGCG and ECG (20μM)

	% sGAG released
control	24± 4 *
rhIL-1β/rhTNFα	57±8
rhIL-1β/rhTNFα + 20 μM EGCG	36±4
rhIL-1β/rhTNFα + 20 μM ECG	19±8 *

Human rheumatoid articular cartilage was obtained at surgery and cultured overnight in 5%(v/v) NCS-containing DMEM. Explants were individually transferred to a 96-well plate and cultured for 9 days in serum-free DMEM in the presence or absence of a combination of rhIL-1β (3nM) and rhTNFα (6nM) and also in the presence or absence of the catechins EGCG or ECG at 20 μM. Medium was changed at 3 and 6 days. The degradation of the cartilage proteoglycan was determined by measuring the sGAG released from the explants as a percentage of total sGAG using the DMB assay. * $p<0.05$ when comparing the proteoglycan released from the group cultured with the cytokine combination to the other groups, as determined using the 2-tailed Mann Whitney test for non-parametric data.

Table 4

The inhibition of cartilage proteoglycan degradation in human non-arthritic cartilage explants by EGCG and ECG (20μM)

	% sGAG released
control	12±2
rhIL-1β/rhTNFα	16±2
rhIL-1β/rhTNFα + 20 μM EGCG	12±2
rhIL-1β/rhTNFα + 20 μM ECG	4±2 **

Human non-rheumatoid articular cartilage was obtained from a patient suffering from Marfan's Syndrome at surgery, and cultured overnight in 5%(v/v) NCS-containing DMEM. Explants were individually transferred to a 96-well plate and cultured for 9 days in serum-free DMEM in the presence or absence of a combination of rhIL-1β (3nM) and rhTNFα (6nM) and also in the presence or absence of the catechins EGCG or ECG at 20 μM. Medium was changed at 3 and 6 days. The degradation of the cartilage proteoglycan was determined by measuring sGAG released from the explants as a percentage of total sGAG using the DMB assay; ** $p<0.005$ when comparing the proteoglycan released from the group cultured with the cytokine combination to the other groups, as determined using the 2-tailed Mann Whitney test for non-parametric data.

Table 5

Time-course for type II collagen degradation in rhIL1α-stimulated bovine nasal cartilage explants treated with or without IL1α and EGCG or ECG.

Treatment	Days							
	0-3	0-7	0-10	0-14	0-17	0-21	0-24	0-28
	% Release of type II collagen							
CONTROL	1±1*	0±8	.0±9	0±7*	0±5**	0±6***	0±6***	0±5***
IL-1	3±1	4±1	5±1	9±3	14±6	34±8	47±8	90±6
IL-1 + EGCG (20μM)	1±1*	2±1	2±1*	3±1*	4±1*	12±5**	19±7*	29±10***
IL-1 + ECG (20μM)	4±2	6±2	16±4*	26±8	9±10	32±10	37±12	41±11

Bovine nasal cartilage explants were cultured for 28 days in the presence or absence of rhIL1α (4.5nM) and the catechins EGCG or ECG (20μM). Medium was changed twice a week. The degradation of type II collagen was measured using the CB11B inhibition ELISA and the data were expressed as the cumulative release of type II collagen. * $p<0.05$, ** $p<0.05$ and *** $p<0.005$ as determined by the Mann Whitney U test for 2-tailed, non-parametric data and compared to the group cultured in the presence of IL1α alone. Data relate to 2 animals, n=6/group/animal.

Table 6

The inhibition at day 28 of type II collagen degradation in rhIL1α-stimulated bovine nasal cartilage explants by EGCG, ECG, EC and EGC.

(i) The % release of type II collagen after 28 days of culture from explants cultured in the presence of 20μM EGCG or ECG.

	% release of type II collagen
control	-1± 4 ***
rhIL-1α	95± 6
rhIL-1α + 20μM EGCG	31±10 ***
rhIL-1α + 20μM ECG	35±10 ***

(ii) The % release of type II collagen after 28 days in culture from explants cultured in the presence of 20μM EC or EGC.

	% release of type II collagen
control	0± 0 ***
rhIL-1α	83± 6
rhIL-1α + 20μM EC	68±11
rhIL-1α + 20μM EGC	33±10 ***

Table 6: Bovine nasal cartilage explants were cultured for 28 days in the presence or absence of rhIL-1α (4.5nM) and the catechins EGCG, ECG, EC or ECG (20μM). Medium was changed twice a week. The degradation of cartilage type II collagen was measured using the CB11B assay and the data are expressed as the cumulative release of type II collagen. * $p<0.05$, ** $p<0.05$ and *** $p< 0.005$ as determined by the Mann Whitney U test for 2-tailed, non-parametric data and compared to the group cultured in the presence of rhIL-1α alone. Data in each table relates to the results obtained from 4 animals, n=14/group in total.

Table 7

Lactate levels in the conditioned media of bovine nasal explants cultured with or without rhIL1α and EGCG or ECG for 28 days.

	μg lactate/explant	
Treatment	**17-20d media**	**24-28d media**
Control	**448±100**	**241±20**
IL-1	**443±56**	**193±20**
IL-1 + EGCG	**300±48**	**194±20**
IL-1 + ECG	**409±70**	**161±21**

Bovine nasal cartilage explants were cultured for a 28 day period in the presence or absence of rhIL1α and the catechins EGCG and ECG at 20μM. Medium was changed twice a week and stored at -20^0C awaiting assay. Lactate levels in the 17-20 day and 24-28 day medium was determined using a kit from Sigma. The data relate to two animals, n=6/animal. The Mann Whitney U test for 2-tailed, non-parametric data was performed, with all groups being compared to the group cultured in the presence of IL-1α. No significant differences were observed.

Table 8

The inhibition by EGCG of TNFα production by human peripheral blood cells.

(i) R&D Systems Kit

	pg/ml TNFα
Control	**52± 8**
Control + 20μM EGCG	**41± 7**
LPS	**476±17**
LPS + 20μM EGCG	**161± 7** ***

(ii) Diaclone Research Kit

	pg/ml TNFα
Control	**nd**
Control + 20μM EGCG	**nd**
LPS	**71±7**
LPS + 20μM EGCG	**50±8**

Human peripheral blood was diluted 1:6 with serum-free DMEM and then incubated for 4 hours at 37°C in a water bath in the presence or absence of bacterial lipopolysaccharide (LPS) (1μg/ml) and EGCG (20μM). The blood was freeze-thawed thrice and the levels of TNFα were quantified using an ELISA kit. ***p< 0.0005, nd = not detectable.

CLAIMS

1. The use of at least one catechin in the manufacture of a medicament for the treatment of arthritis.

2. The use according to Claim 1 wherein said catechin is selected from:(+)epicatechin; (+) catechin; (-)epicatechin;(-) catechin; (-) epigallocatechin;(-) gallocatechin; (-)epicatechin gallate; (-) catechin gallate; (-) epigallocatechin gallate; (-) gallocatechin gallate.

3. The use of a catechin according to claim 1 or 2 wherein said catechin is epigallocatechin gallate.

4. The use of a catechin according to claim 1 or 2 wherein said catechin is epicatechin.

5. The use according to any of claims 1-4 wherein said arthritic condition is selected from: osteoarthritis; rheumatoid arthritis; inflammatory arthritis; osteochondritis; acute pyrophosphate arthritis; reactive arthritis; psoriatic arthritis; juvenile arthritis; lupus erythematosus; Sjögren's syndrome; relapsing polychondritis; ankylosing spondylitis; psoriatic arthritis; MSUM (gout); CPPD (psuedogout, chondrocalcinosis); chondrolysis; bursitis.

6. The use according to claim 5 wherein said medicament is for the treatment of osteoarthritis.

7. The use according to claim 5 wherein said medicament is for the treatment of rheumatoid arthritis.

8. The use according to any of claims 1-7 wherein said medicament is for the prophylactic treatment of arthritis.

9. The use according to claim 8 wherein said prophylactic treatment is for animals with a genetic predisposition to arthritis.

10. The use according to claim 8 wherein said prophylactic treatment is to protect animals with an increased probability of developing arthritis due to joint damage.

11. A therapeutic composition comprising at least one catechin and at least one anti-arthritic agent or biopolymer.

12. A therapeutic composition according to claim 11 wherein said anti-arthritic agent is hyaluronic acid.

13. A therapeutic composition according to Claim 11 wherein said anti-arthritic agent is glucosamine.

14. A therapeutic composition according to claim 13 wherein glucosamine is g glucosamine sulphate.

15. A therapeutic composition according to any of Claims 11 - 14 wherein said c composition is immune silent.

16. A therapeutic composition according to any of claims 11-15 wherein said catechin and said anti-arthritic agent are administered as an admixture.

17. A therapeutic composition according to any of claims 11-15 wherein said catechin and said anti-arthritic agent are cross-linked, coupled or associated together.

18. A therapeutic composition according to claim 17 wherein said catechin is cross-linked, coupled or associated with hyaluronic acid.

19. A method to cross-link or couple at least one catechin to at least one anti-arthritic agent comprising:

i) providing at least one catechin and at least one anti-arthritic agent;
ii) providing conditions conducive to the cross-linking or coupling of said catechin to said agent; and optionally
iii) purifying the cross-linked or coupled complex from the reaction mixture.

20. A method according to claim 19 wherein said anti-arthritic agent is hyaluronic acid and said catechin is selected from: (+)epicatechin; (+) catechin;(-) epicatechin;(-)catechin;(-)epigallocatechin;(-)gallocatechin;(-)epicatechin gallate; (-) catechin gallate; (-) epigallocatechin gallate;(-) gallocatechin gallate.

21. A method for treating an arthritic condition comprising administering to an animal a pharmacologically effective amount of the medicament according to any of Claims 1- 4 or a therapeutic composition according to any of Claims 11- 18.

22 A method according to Claim 21 wherein said arthritic condition is selected from: osteoarthritis; rheumatoid arthritis; inflammatory arthritis; osteochondritis; acute pyrophosphate arthritis; reactive arthritis; psoriatic arthritis; juvenile arthritis; lupus erythematosus; Siögren's syndrome; relapsing polychondritis; ankylosing spondylitis; psoriatic arthritis; MSUM (gout); CPPD (psuedogout, chondrocalcinosis); chondrolysis; bursitis.

22. A method of treating an arthritic condition according to claims 21 or 22 wherein said arthritic condition is osteoarthritis.

23. A method of treating an arthritic condition according to claims 21 or 22 wherein said arthritic condition is rheumatoid arthritis.

24. A method to screen for agents with anti-arthritic properties.

25. A method according to claim 24 wherein said method comprises:

i) providing a cartilage sample;
ii) addition of an effective amount of at least one agent to be tested;
iii) addition of at least one pro-inflammatory cytokine; and
iv) monitoring at least one molecule indicative of cartilage breakdown.

26. A method according to claim 25 wherein said pro-inflammatory cytokine is selected from: interleukin-1α; interleukin-1β; oncostatin M; tumour necrosis factor α.

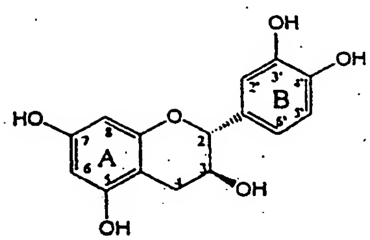
27. A method according to claim 24 wherein said method comprises:

i) providing a cartilage sample;
ii) addition of an effective amount of at least one agent to be tested;
iii) addition of at least one vitamin A metabolite; and
iv) monitoring at least one molecule indicative of cartilage breakdown.

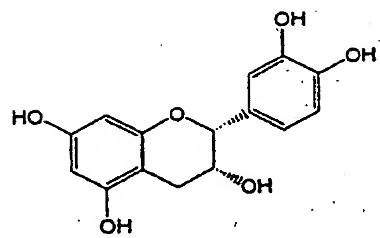
28. The method according to claim 27 wherein said vitamin A metabolite is all trans-retinoic acid.

29. An agent identified by the screening methods of claims 24-28.

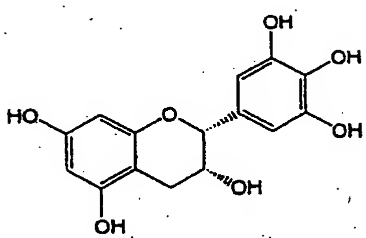
Figure 1a



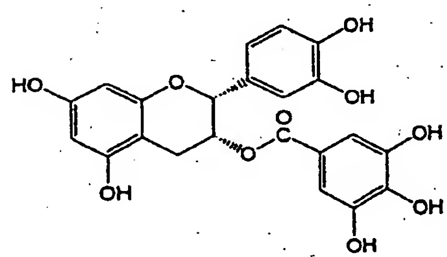
(+)-catechin (CAT)



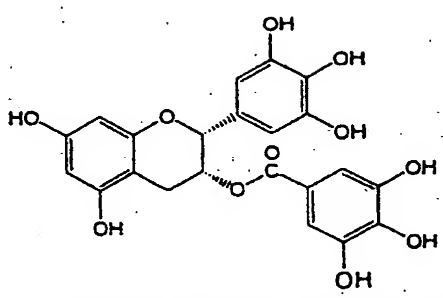
(-)-epicatechin (EC)



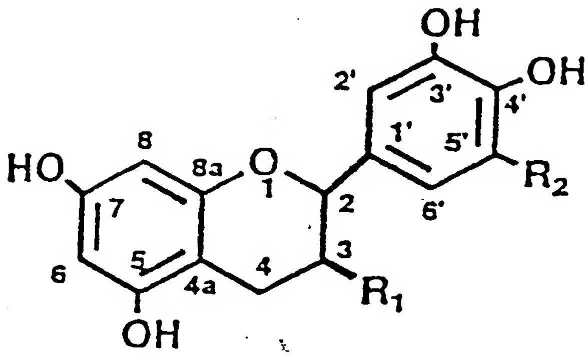
(-)-epigallocatechin (EGC)

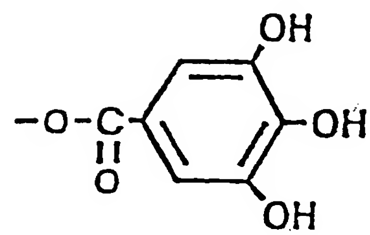


(-)-epicatechin gallate (ECG)



(-)-epigallocatechin gallate (EGCG)



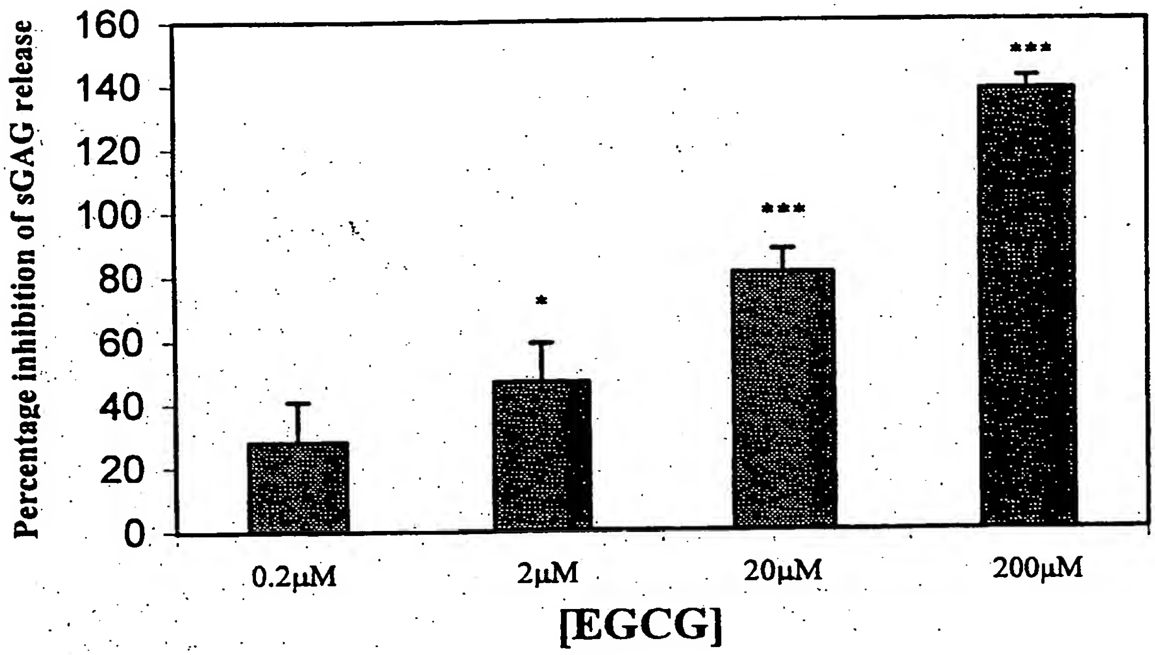


Galloyl group (GA)

Catechins	Configuration	R1	R2	SC_{50} (μM)
(+)-Epicatechin	2S, 3R	OH	H	2.9
(+)-Catechin	2R, 3S	OH	H	2.9
(−)-Epicatechin	2R, 3R	OH	H	3.0
(−)-Catechin	2S, 3R	OH	H	2.7
(−)-Epigallocatechin	2R, 3R	OH	OH	1.8
(−)-Gallocatechin	2S, 3R	OH	OH	2.1
(−)-Epicatechin gallate	2R, 3R	GA	H	1.2
(−)-Catechin gallate	2S, 3R	GA	H	1.4
(−)-Epigallocatechin gallate	2R, 3R	GA	OH	1.2
(−)-Gallocatechin gallate	2S, 3R	GA	OH	1.1
α-Tocopherol (Vitamin E)				18
Ascorbic acid (Vitamin C)				13

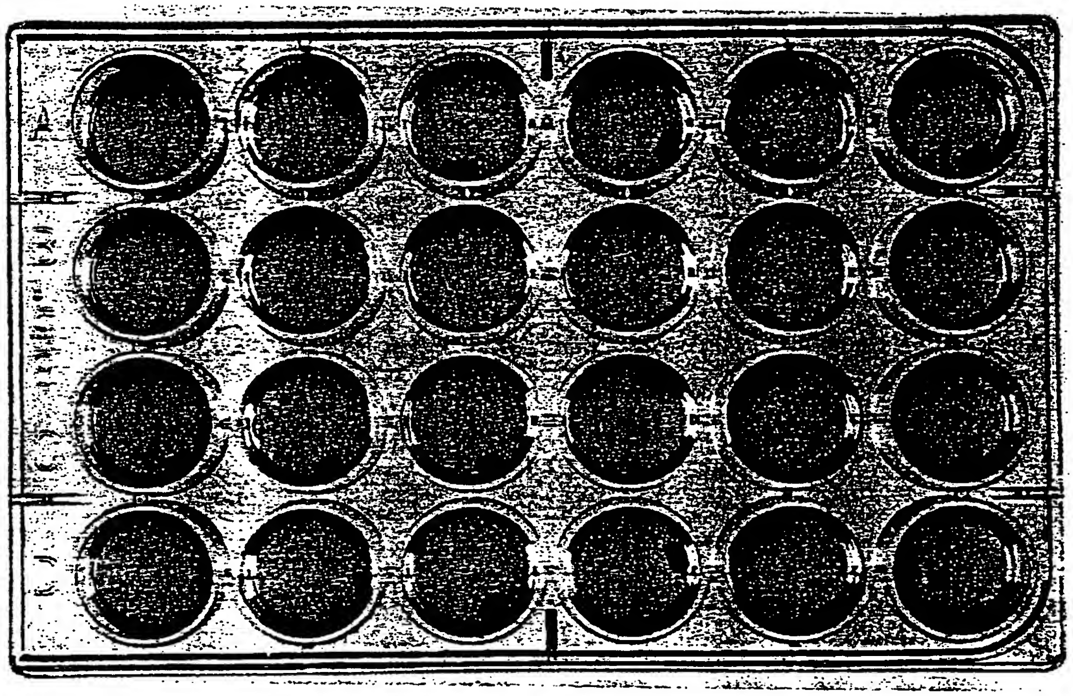
Figure 1b

Figure 2



Bovine nasal cartilage explants were cultured for 5 days in serum-free DMEM in the presence or absence of rhTNFα (3nM) and in the presence or absence of 0.2-200μM EGCG. Medium was changed on day 3.

Figure 3



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 December 2000 (14.12.2000)

PCT

(10) International Publication Number
WO 00/74662 A3

(51) International Patent Classification[7]: A61K 31/35, A61P 19/02

(21) International Application Number: PCT/GB00/02048

(22) International Filing Date: 6 June 2000 (06.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/137,699 7 June 1999 (07.06.1999) US
0007321.3 27 March 2000 (27.03.2000) GB

(71) Applicant (*for all designated States except US*): UNIVERSITY OF SHEFFIELD [GB/GB]; Western Bank, Sheffield S10 2TN (GB).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): BUTTLE, David [GB/GB]; Unviersity of Sheffield, Medical School, Beech Hill Road, Sheffield S10 2R (GB). ADCOCKS, Clair [GB/GB]; University of Sheffield, Medical School, Beech Hill Road, Sheffield S10 2R (GB). COLLIN, Peter [US/US]; P.O. Box 151, Stonington, ME 04681 (US).

(74) Agent: HARRISON GODDARD FOOTE; Tower House, Merrion Way, Leed LS2 8PA (GB).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report: 14 March 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/74662 A3

(54) Title: ARTHRITIS TREATMENT

(57) Abstract: The invention relates to the use of catechins in the treatment of various forms of arthritis, including the use of combinations of catechins and other anti-arthritic agents, like hyoluronic acid and glucosamine and glucosamine sulfate, in said treatment; medicaments and compositions for use in said treatment; and methods to identify agents with anti-arthritic properties.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/02048

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/35 A61P19/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, EMBASE, BIOSIS, CHEM ABS Data, WPI Data, MEDLINE, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 2 306 321 A (CHILD ANNE) 7 May 1997 (1997-05-07) abstract; claims 1-5 ---	1,2, 5-10, 21-23,29
X	BE 886 568 A (CONTINENTAL PHARMA) 9 June 1981 (1981-06-09) page 2, line 1-10 page 2, line 26 -page 5, line 28; tables 1,2 page 8, line 1 -page 10, line 32 ---	1,2, 5-11, 21-23,29
X	US 4 268 517 A (NIEBES PAUL J ET AL) 19 May 1981 (1981-05-19) abstract; claims 1-4 column 1, line 67 -column 2, line 5 ---	1,2, 5-11, 21-23,29

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 July 2001	10.08.01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	A. Jakobs

5

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 742 012 A (KUREHA CHEMICAL IND CO LTD) 13 November 1996 (1996-11-13) abstract page 2, line 9-13 page 3, line 1-6 page 5, line 26 -page 6, line 54; claims 1,6,7; example 4 ---	1-11, 21-23,29
X	WO 96 28178 A (INDENA SPA ;BOMBARDELLI EZIO (IT); MORAZZONI PAOLO (IT); MUSTICH G) 19 September 1996 (1996-09-19) abstract page 5, line 19-24 page 8, line 27 -page 9, line 4; claims 13,14,21 ---	1,2, 5-11, 21-23,29
X	HAQQI T M ET AL: "PREVENTION OF COLLAGEN-INDUCED ARTHRITIS IN MICE BY A POLYPHENOLIC FRACTION FROM GREEN TEA" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,NATIONAL ACADEMY OF SCIENCE. WASHINGTON,US, vol. 96, no. 8, 13 April 1999 (1999-04-13), pages 4524-4529, XP000961089 ISSN: 0027-8424 abstract page 4524, column 2, paragraph 3; table 1 page 4529, column 1, paragraphs 2,3 ---	1,2, 5-10, 21-23,29
X,P	AKIZAWA ET AL: "Catechin derivatives as matrix metalloprotease (MMP) inhibitors for treatment of MMP-related diseases" CHEMICAL ABSTRACTS + INDEXES,AMERICAN CHEMICAL SOCIETY. COLUMBUS,US, vol. 133, no. 13, 2000, XP002154308 ISSN: 0009-2258 abstract ---	1
X	NIEBES P ET AL: "EFFECT OF DRUGS ON COLLAGEN FIBRILLATION AND COLLAGEN DEGRADATION IN VITRO" EUROPEAN JOURNAL OF RHEUMATOLOGY AND INFLAMMATION,GB,CAMERON PUBLISHERS, CASTLETOWN, vol. 2, no. 2, 1979, pages 226-229, XP000614080 ISSN: 0104-1610 page 226, paragraph 1; table 1 page 228, paragraphs 3,4 ---	1,2, 5-10, 21-23,29

-/--

5

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/02048

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AUCAMP J ET AL: "INHIBITION OF XANTHINE OXIDASE BY CATECHINS FROM TEA (CAMELLIA SINENSIS)" ANTICANCER RESEARCH,HELENIC ANTICANCER INSTITUTE, ATHENS,,GR, vol. 17, no. 6D, November 1997 (1997-11), pages 4381-4385, XP000925670 ISSN: 0250-7005 the whole document page 4384, column 1	1-10, 21-23,29
X	CARNEY S.L. ET AL: "Anthraquinones related to rhein inhibit glucose uptake into chondrocytes, a mechanism for anti-osteoarthritis drugs?." BIOORGANIC AND MEDICINAL CHEMISTRY LETTERS, (1997) 7/7 (817-822)., XP004136136 abstract; table 1	1,2, 5-10, 21-23,29
X	SHEU SHIOW-YUN ET AL: "Superoxide anion scavenge effect of Quercus glauca Thunb. in whole blood of patients with ankylosing spondylitis." AMERICAN JOURNAL OF CHINESE MEDICINE, vol. 25, no. 3-4, 1997, pages 307-315, XP000981297 ISSN: 0192-415X abstract	1,2, 5-10, 21-23,29
X	RAO, C. N. ET AL: "Influence of bioflavonoids on the collagen metabolism in rats with adjuvant induced arthritis" ITAL. J. BIOCHEM. (1981), 30(1), 54-62, XP000981289 abstract; tables 1-3	1,2, 5-10, 21-23,29
X	SWARNALAKSHMI T ET AL: "ANTI INFLAMMATORY ACTIVITY OF LEVO EPI CATECHIN A BIO FLAVONOID ISOLATED FROM ANACARDIUM-OCCIDENTALE" INDIAN JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 43, no. 6, 1981, pages 205-208, XP000981290 ISSN: 0250-474X abstract	1,2, 5-10, 21-23,29

-/--

5

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	RAO C N ET AL: "BIO FLAVONOID MEDIATED STABILIZATION OF COLLAGEN IN ADJUVANT INDUCED ARTHRITIS" SCANDINAVIAN JOURNAL OF RHEUMATOLOGY, vol. 12, no. 1, 1983, pages 39-42, XP000981292 ISSN: 0300-9742 abstract page 39, column 1 -column 2, paragraph 1 page 41, column 1, paragraph 1 ---	1,2, 5-10, 21-23,29
X	BLUMENKRANTZ N ET AL: "EFFECTS OF SOME CONNECTIVE TISSUE ACTIVE DRUGS ON PROTO COLLAGEN PROLINE HYDROXYLASE ACTIVITY" SCANDINAVIAN JOURNAL OF RHEUMATOLOGY, vol. 7, no. 2, 1978, pages 123-127, XP000981242 EN ISSN: 0300-9742 abstract page 123, column 1, paragraphs 1,2 ---	1,2, 5-10, 21-23,29
E	DATABASE WPI Section Ch, Week 200059 Derwent Publications Ltd., London, GB; Class B04, AN 2000-614836 XP002169737 & JP 2000 226329 A (MEIJI MILK PROD CO LTD), 15 August 2000 (2000-08-15) abstract ---	1,5-10, 21-23
X	PATENT ABSTRACTS OF JAPAN vol. 1995, no. 11, 26 December 1995 (1995-12-26) & JP 07 223941 A (NIPPON HAM KK), 22 August 1995 (1995-08-22) abstract ---	1-3, 5-11,15, 21-23
X	DATABASE WPI Section Ch, Week 199712 Derwent Publications Ltd., London, GB; Class B04, AN 1997-126438 XP002169738 & JP 09 009987 A (AMANO PHARM KK), 14 January 1997 (1997-01-14) abstract ---	11,13, 14,17, 19,20
X	PATENT ABSTRACTS OF JAPAN vol. 1998, no. 01, 30 January 1998 (1998-01-30) & JP 09 227374 A (TAIYO KAGAKU CO LTD), 2 September 1997 (1997-09-02) abstract ---	1-10, 21-23

-/--

5

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/02048

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
E	WO 00 47193 A (KAROLINSKA INNOVATIONS AB ;CAO YIHAI (SE)) 17 August 2000 (2000-08-17) abstract page 2, line 14-21 page 5, line 14-21 page 6, line 13 -page 7, line 4; claims 1-9 ---	1-10, 21-23
X	GARIBOLDI EMANUELE ET AL: "LC-UV-electrospray-MS-MS mass spectrometry analysis of plant constituents inhibiting xanthine oxidase." PHARMACEUTICAL RESEARCH (NEW YORK), vol. 15, no. 6, June 1998 (1998-06), pages 936-943, XP001003087 ISSN: 0724-8741 the whole document ---	1-5,21, 22
X,P	ISLAM S ET AL: "INHIBITION OF TNF-ALPHA-INDUCED CASPASE-3 ACTIVITY BY EPIGALLOCATECHIN-3-GALLATE (EGCG) IN HUMAN ARTICULAR CHONDROCYTES" ARTHRITIS AND RHEUMATISM,LIPPINCOTT, PHILADELPHIA,US, vol. 42, no. 9, September 1999 (1999-09), page S92 XP000972239 ISSN: 0004-3591 abstract -----	1-3,5, 21,22

5

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/02048

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 21-23 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: –
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

As a result of the prior review under R. 40.2(e) PCT, no additional fees are to be refunded.

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [X] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
1-3,5-23

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[X] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1,5-19,21-23 relate to a large number of possible compounds (catechins). Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Present claims 11,15-19,21-23 relate to a product/compound/method defined by reference to a desirable characteristic or property, namely
- one anti-arthritic agent
- a biopolymer.

The claims cover all products/compounds/methods having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such products/compounds/methods. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product/compound/method by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the products/compounds/methods specifically mentioned in the claims.

Rem.: there are two claims numbered 22.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1,2,5-23,29 (partially) 4

   Use of (+) epicatechin, (+) catechin, (-) epicatechin or (-) catechin alone or in a composition comprising one catechin and at least one anti-arthritic agent or biopolymer including hyaluronic acid, glucosamine and glucosamine sulphate, in relation to the treatment of arthritis, and methods to cross-link or couple said catechin to said anti-arthritic agent or biopolymer.

2. Claims: 1,2,5-23,29 (partially)

   Use of (-) epigallocatechin, (-) gallocatechin, alone or in a composition comprising one catechin and at least one anti-arthritic agent or biopolymer including hyaluronic acid, glucosamine and glucosamine sulphate, in relation to the treatment of arthritis, and methods to cross-link or couple said catechin to said anti-arthritic agent or biopolymer.

3. Claims: 1,2,5-23,29 (partially)

   Use of (-) epicatechin gallate, (-) catechin gallate alone or in a composition comprising one catechin and at least one anti-arthritic agent or biopolymer including hyaluronic acid, glucosamine and glucosamine sulphate, in relation to the treatment of arthritis, and methods to cross-link or couple said catechin to said anti-arthritic agent or biopolymer.

4. Claims: 1,2,5-23,29 (partially), 3

   Use of (-) epigallocatechin gallate, (-) gallocatechin gallate alone or in a composition comprising one catechin and at least one anti-arthritic agent or biopolymer including hyaluronic acid, glucosamine and glucosamine sulphate, in relation to the treatment of arthritis, and methods to cross-link or couple said catechin to said anti-arthritic agent or biopolymer.

5. Claims: 24-28

   A method to screen for agents with anti-arthritic properties comprising providing a cartilage sample, addition of at least one agent to be tested, addition of at least one pro-inflammatory cytokine or vitamin A metabolite, and monitoring at least one molecule indicative of cartilage breakdown.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 00/02048

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2306321 A	07-05-1997	NONE	
BE 886568 A	09-06-1981	NONE	
US 4268517 A	19-05-1981	NONE	
EP 0742012 A	13-11-1996	JP 8301781 A	19-11-1996
		JP 8301757 A	19-11-1996
		JP 2892300 B	17-05-1999
		JP 8301784 A	19-11-1996
		JP 3003978 B	31-01-2000
		JP 9012459 A	14-01-1997
		JP 9040556 A	10-02-1997
		JP 2933511 B	16-08-1999
		JP 9040553 A	10-02-1997
		AU 689036 B	19-03-1998
		AU 5214096 A	19-12-1996
		CA 2175985 A	11-11-1996
WO 9628178 A	19-09-1996	IT MI950493 A	16-09-1996
		AU 696676 B	17-09-1998
		AU 5004096 A	02-10-1996
		CA 2215210 A	19-09-1996
		EP 0814823 A	07-01-1998
		JP 10512582 T	02-12-1998
		NO 974188 A	11-09-1997
		US 6096359 A	01-08-2000
		US 5989557 A	23-11-1999
JP 2000226329 A	15-08-2000	NONE	
JP 07223941 A	22-08-1995	NONE	
JP 9009987 A	14-01-1997	NONE	
JP 09227374 A	02-09-1997	NONE	
WO 0047193 A	17-08-2000	AU 2567300 A	29-08-2000